Remarks

Claims 1-4, 22-26, 41, 42 and 59-64 were pending. Claims 66-71 were added. No claims were cancelled. Therefore, claims 1-4, 22-26, 41, 42 and 59-71 are now pending.

Telephone Interview Summary

Applicants thank Examiner Marvich and her supervisor Examiner Leffers for the courtesy of a telephone interview with Applicants' representative Sheree Lynn Rybak, Ph.D. on July 20, 2004. During this interview, the 35 U.S.C. § 112, first paragraph rejections were discussed.

Specifically, with respect to the written description requirement, it was agreed that Applicants would direct the examiner to the places in the application describing the EDA1-II protein structural and functional information sufficient to describe the genus. With respect to the enablement requirement, Applicants discussed the Gaide and Schneider (*Nature Med.* 9:614, 2003) reference and explained that the EDA1-II protein referred to in the application is the same as the EDA1 protein referred to in Gaide and Schneider, and explained that the Tabby mouse is the accepted model for human ectodermal dysplasia.

Support for new claims

Support for the new claims can be found throughout the specification, for example:

Claim 65: page 21, lines 21-25

Claim 66: page 21, lines 25-26

Claim 67: page 8, lines 8-9; page 21, lines 21-22

Claim 68: page 21, lines 21-22

Claim 69: page 21, lines 14-16

Claims 70-71: page 22, lines 12-15 and FIG. 4

Claim Objections

Claims 2-4 and 42 were objected to due to informalities. Claims 2-4 now include the word "the" prior to the phrase "method is a method of" and claim 42 now spells-out the XLHED and HED abbreviations. In view of these amendments, Applicants request that the objections to the claims be withdrawn.

35 U.S.C. § 112, first paragraph, written description

Claims 59-64 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Applicants respectfully disagree and request reconsideration. It is asserted that Applicants only disclose SEQ ID NO: 2 (EDA1-II) and three fragments thereof, and are therefore not entitled to other fragments, fusion proteins, and mutants of SEQ ID NO: 2 with 1-10 amino acid substitutions.

The specification provides a detailed correlation between the structure of EDA1-II and the ability to induce development of ectodermal structures such as hair follicles, teeth and sweat glands. For example, Example 2 of the application (pages 20-22) provides details on the structure of EDA1-II, including which regions of the protein are most likely to have the desired biological activity, and which particular amino acids are believed to be important for the desired biological activity. The importance of the C-terminus is described. As noted on page 21, lines 6-9, the presence of three cystine residues in the C-terminal domain of human EDA1-II and its mouse homolog Tabby indicates that EDA1-II function can be interfered with by removing the C-terminal amino acids 153-239. In addition, a sequence comparison of EDA1-II and Tabby demonstrates that there is only a single, conservative amino acid substitution in the terminal 211 amino acids, indicating that the that region is important in protein function (see page 21, lines 14-17 and FIG. 1). The application states that "The biologically active domains of the EDA1-II protein are within approximately the C-terminal 240 amino acids..." (page 21, lines 21-22). Additional domains are described on page 20, lines 33-37. Because the application describes in detail the location of the EDA1-II domains, and describes which are important for EDA1-II biological activity, one skilled in the art can determine which fragments, fusions, and variants of EDA1-II would have the desired biological activity.

In addition, the application provides particular teachings as to which amino acids of EDA1-II can be substituted, without substantial loss of biological activity. For example, page 21, lines 17-20 of the specification lists specific regions (such as the region including amino acids 1-180 and particular sub-regions thereof) which are less conserved between EDA1-II and the Tabby homolog (also see alignment shown in FIG. 1). Those skilled in the art will appreciate that less conserved regions are more likely to tolerate substitutions than are regions that are highly conserved. Further information on amino acid substitutions that can be made to EDA1-II

are provided on page 22, lines 8-17 and FIG. 4. An alignment of the central beta sheet region of EDA1-II with other TNF family members identifies those amino acids that are highly conserved (dots above the column in FIG. 4), and which are not. The specification notes that "the second amino acid residue (E) [of the central beta sheet] may be substituted with V, L, A, or T...while retaining biological activity" (page 22, lines 12-15). Based on the detailed description in the application describing the regions of conservation between human EDA1-II and its mouse homolog Tabby, one skilled in the art would appreciate that regions that are less conserved would more likely tolerate amino acid substitutions (such as the N-terminal 180 amino acids) than would regions that are highly conserved, such as the C-terminus.

Information on mutations in EDA1-II present in humans suffering from X-linked hypohidrotic ectodermal dysplasia (XLHED) is provided in Example 4 (pages 23-25) and FIG. 6. The functional effect of these mutations is provided in Example 5 (pages 25-26). For example, several specific amino acid substitutions in EDA1-II resulting from the DNA changes are listed in Table 1 on page 24. The location of these mutations is correlated with particular domains of EDA1-II in Example 5, such as the collagen domain. Therefore, the mutations shown in Table 1 lead to decreased growth of ectodermal structures. In view of this information, those skilled in the art will understand that incorporation of one or more of the mutations shown in Table 1 into EDA1-II (such as SEQ ID NO: 2) would likely not stimulate ectodermal structures (in contrast to the purpose of the present claims which are directed to increasing growth of one or more ectodermal structures), and instead would likely decrease the presence of ectodermal structures.

The application also provides detailed information on how to test EDA1-II variant sequences for their ability to stimulate hair, tooth, or skin growth (for example see Example 19 starting on page 50). This information, in combination with the detailed correlation between the structure of the variant EDA1-II sequences and its biological activity, provides those skilled in the art with the information they need to identify those species encompassed by the present disclosure.

Further evidence that those skilled in the art can identify fragments of EDA1-II that will have the desired biological activity is demonstrated by the results presented in Gaide and Schneider (*Nature Med.* 9:614, 2003) cited in the Office action. Gaide and Schneider demonstrate that administration of the C-terminal 147 amino acids of human EDA1-II (referred to therein as EDA1) to Tabby mice (the mouse model for human ectodermal dysplasia) was able

to rescue the Tabby phenotype by promoting the development of sweat glands, hair and teeth (see FIG. 2). These results demonstrate that those skilled in the art can identify functional EDA1-II fragments and fusion proteins that have the desired biological activity. Furthermore, this data confirms the teaching provided in the application that the C-terminus of EDA1-II is important for development of ectodermal structures.

Similarly, Yan *et al.* (*Science*, 290:523-7, 2000; a copy of which is enclosed) demonstrated that recombinantly expressed and purified amino acids 179-391 of EDA1-II protein (referred to in Yan *et al.* as EDA-A1), and a variant thereof (EDA-A2), stimulate hair follicle development in a skin organ culture system (see FIG. 4D of Yan *et al.*). These results support the Applicant's conclusion that EDA1-II can promote the development of hair follicles, and confirms the teaching provided in the application that the C-terminus of EDA1-II is important for development of ectodermal structures.

Because Applicants have provided a detailed correlation between the structure of EDA1-II and the ability to induce development of ectodermal structures such as hair follicles, teeth and sweat glands, as well as information on how to identify EDA1-II fragments and variants that have the desired biological activity, Applicants request that the 35 U.S.C. § 112, first paragraph rejection of claims 59-64 be withdrawn.

35 U.S.C. § 112, first paragraph, enablement

Claims 1-4, 22-26, 41-42 and 59-64 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Office action recites the factors from *In re Wands*, and concludes that it would require undue experimentation for one skilled in the art to make and used the claimed invention based on the disclosure and information known in the art. Applicants respectfully disagree and request reconsideration.

Nature of the invention: Applicants agree that the nature of the currently pending claims is a method of increasing one or more of hair follicle development, tooth development, or sweat gland development, in a tissue, by increasing EDA1-II activity in the tissue.

Scope of the invention: The method recites administration of EDA1-II proteins (as well as variants, fragments, and fusions thereof having EDA1-II biological activity) to subjects having an ectodermal disorder.

Number of working examples and guidance: It is incorrectly stated on page 6 of the Office action that there are no proposed methods for the application of the methods for human use. In addition, there is teaching throughout the specification describing how the EDA1-II protein (and variants, fragments, and fusions thereof) can be administered *in vivo* to animals or humans.

The present disclosure is not limited to administration of EDA1-II proteins to mice, and indeed includes administration to humans. The examples that particularly describe the use of mice provide particular teaching to those skilled in the art how to *test* EDA1-II variants, fragments, or fusions, to determine if such proteins have the desired activity, prior to administration of such a protein to a human subject. However these examples provide guidance and teaching to those skilled in the art for how to administer EDA1-II proteins to any subject, including a human subject.

It appears that the reference on page 5 of the Office action when referring to page 28 is referring to page 50 of the application as filed (Example 19). The Office action states particular examples of administration to mice, and then concludes that there are not proposed methods for application of the recited methods for human use. This conclusion is incorrect. Clear teaching is provided throughout the application for administration of EDA1-II proteins to treat ectodermal disorders in humans. Even the Example referred to in the Office action includes specific teachings for administration to humans and treatment of ectodermal disorders. For example, page 50, lines 28-32 states that "[v]arious methods available for the appropriate delivery of the protein to hair follicles in *human skin* can be performed...." (emphasis added), and then lists four references that provide such information (page 50, lines 29-31). Because methods of administration of proteins to human skin, including the scalp are known by those skilled in the art, there is no duty on the part of the Applicant to repeat in the application that which is already known in the art. Evidence that those in the art know how to administer proteins to humans to stimulate hair growth are provided in the references cited in the application. The cited Hoffman reference (*J. Drug Target*, 5:67-74, 1998) teaches the use of liposomes to deliver small molecules such as proteins and nucleic acids by topical application to the human scalp. Lieb *et al.* (*J. Pharm. Sci.* 86:1022-9, 1997) teaches methods that can be used to deliver large molecules to follicles of human scalp skin. Lauer *et al.* (*Pharm. Res.* 12:179-86, 1995) provide a review of

methods that can be used for follicular drug delivery. Illel (*Crit. Rev. Ther. Drug. Carrier Syst.* 14:207-19, 1997) disclose recent formulation methodologies that allow administration of a drug product through the follicular pathway. Example 19 also particularly teaches "topical daily application to a bald area of the *human* scalp." (page 50, line 34, emphasis added).

Example 19 also discloses methods to increase tooth growth, which are not limited to application in mice. For example, on page 51, lines 1-12, discloses administration of an EDA1-II protein (at 1 ng/ml – 1 g/l) to an in vitro tooth organ culture system, and then "subsequent introduction of teeth into *humans* or other organisms." (page 51, line 12, emphasis added).

Methods of increasing sweat gland development in a human are also disclosed in Example 19 on page 51, lines 13-24. This example discloses that sweat gland development can be stimulated "in individuals for who the normal sweating mechanisms is compromised by disease, trauma, burns or surgery." (page 51, lines 14-15). As defined on page 9, lines 18-19, "'individual' includes both human and veterinary subjects." Therefore, this example includes methods of increasing sweat gland development in a human subject. Although injection into the footpads of mice is particularly discussed as a method of testing the ability of EDA1-II proteins to have such activity, one skilled in the art would appreciate that injection into the skin of a human can also be performed, especially as the application discloses injection of EDA1-II proteins into humans (for example see page 72, lines 21-23).

Methods of increasing epidermal growth in a human are also disclosed in Example 19 on page 51, line 25 – page 52, line 2. This example discloses that epidermal growth can be stimulated "in cases involving trauma or burns." (page 51, line 27). Because this example is not limited to the treatment of any particular subject, it includes methods of increasing epidermal growth in a human subject. Although injection into the wound of a mouse is particularly discussed as a method of *testing* the ability of EDA1-II proteins to have such activity, one skilled in the art would appreciate that injection into the skin of a human can also be performed, especially as the application discloses injection of EDA1-II proteins into humans (for example see page 72, lines 21-23).

Additional teachings throughout the specification demonstrate that the teachings can be applied to the treatment of a human having an ectodermal disorder. For example, on page 14 of the application, a therapeutically effective amount of EDA1-II protein is described as an amount effective to achieve a desired effect in a *subject* being treated (page 14, lines 31-32, emphasis

added), such as an increase the growth and/or development of hair follicles, teeth, sweat glands and/or any tissue of ectodermal origin in a tissue, such as a tissue in a *subject* to who it is administered (see page 14, lines 21-25, emphasis added). Subject is defined in the application to include "*human* and veterinary subjects" (page 14, lines 17-18, emphasis added). The application further states that the EDA1-II proteins "have equal application in medial and veterinary settings." (page 15, lines 13-14) Therefore, the present application discloses administration of EDA1-II variants, fragments, or fusions proteins to treat an ectodermal disease in a human subject.

Further evidence that the specification teaches administration of an EDA1-II protein (or variants, fragments, or fusions thereof), is provided in the language that describes "improv[ing] signs and/or symptoms [of] a disease such as HED, for example by increasing the growth and/or development of hair follicles, teeth, sweat glands and/or any tissue of ectodermal origin." (page 14, lines 34-36). HED, hypohidrotic ectodermal dysplasia is a human disorder. The mouse homolog of this disorder is referred to as Tabby (see page 2, lines 12-13).

Dosages of proteins (0.01 mg/kg to about 1 g/kg body weight) that can be administered to a human subject are provided on page 15, lines 4-12. The teachings in this paragraph are not limited to a particular organism, and can thus be applied to human or other subjects.

Information on how to administer EDA1-II to human subjects is provided throughout the application. For example, pages 16, line 21 – page 19, line 3 provides details on how to treat an ectodermal disorder, such as "increasing hair follicle development, tooth development, or sweat gland development in a tissue by increasing EDA1-II activity in the tissue." (page 16, lines 29-30). Particular examples of administration of EDA1-II proteins are discussed on page 17, lines 12-20, and particular modes of administering the protein (including topical administration and injection of proteins) are discussed on page 72, lines 18-37. For example, the disclosure teaches that "[m]ethods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes." (page 72, lines 21-23). "In one embodiment, pharmaceutical compositions disclosed herein are delivered locally to the area in need of treatment, for example by...topical applications...by injection...[or] by direct administration at a site where hair growth, tooth growth, epithelial, or sweat gland growth is desired." (page 72, lines 30-35). The pharmaceutical compositions of the present application "include a therapeutically effective amount of the EDA1-II ...protein...." (page 73, lines 10-11).

In summary, the present application provides sufficient teachings to enable one skilled in the art to treat ectodermal disorders in humans, including HED, by administering an EDA1-II peptide (including variants, fragments, or fusions thereof).

State of the art: Based on the teachings in the application, and the work of Gaide and Schneider, the state of the art of protein therapeutics is sufficient that one skilled in the art would recognize that the technique provided in the specification enable one to make and use the claimed invention.

Although the Office action notes that Torchilin and Lukyanvoe teach that there are unsolved problems with rapid elimination of proteins though renal filtration, it appears that such problems do not occur with the EDA1-II proteins. As disclosed in Gaide and Schneider, intradermal injection of an EDA1-II fragment (referred to therein as EDA1) was able to rescue the defects in Tabby mice, the mouse model for human ectodermal dysplasia. Therefore, EDA1-II (and variants, fragments, or fusions thereof) does not appear to be rapidly eliminated from the circulation, or at least is present for a time long enough to achieve the desired effect on ectodermal structures.

Even assuming that EDA1-II (or variants, fragments, or fusions thereof) were rapidly eliminated from the body, such problems would not be expected to be encountered when the protein is administered topically. As disclosed throughout the application, topical administration is one method that can be used to deliver an EDA1-II protein (for example, see page 21, line 31; page 50, line 34; and page 72, line 31) to a subject having an ectodermal disorder. However, as already noted, systemic administration is fully disclosed in the specification and its usefulness demonstrated by Gaide and Schneider.

In addition, Mrsny (*Expert Opin. Biol. Ther.* 24(1):65-73, 2004; copy enclosed herein) notes that many, if not most, potential protein therapeutics act at the site where administered. Therefore, application of an EDA1-II protein to an area in need of treatment, such as a wound, scalp or tooth, is likely to act at that area.

In summary, based on the recent results presented in Gaide and Schneider, EDA1-II proteins can be delivered to a subject to treat ectodermal disorders, without the problems encountered with other proteins as discussed by Torchilin and Lukyanov.

Unpredictability of the art:

It is concluded in the Office action that the success of animal models cannot be considered as evidence of success of treatment. Applicants disagree and request reconsideration.

The Tabby mouse disclosed throughout the specification, and used by Gaide and Schneider, is the accepted mouse model for the human disease ectodermal dysplasia. As noted in the Gaide and Schneider abstract, "[m]ice with the Tabby phenotype share many symptoms with human XLHED patients because both phenotypes are caused by mutations of the syntenic ectodysplasin A gene (Eda) on the X chromosome." Other groups skilled in the art have also recognized that the *Tabby* gene is the mouse homolog of the human *EDA1-II* gene (for example see Srivastava *et al.*, *Proc. Natl. Acad. Sci. USA* 94:13069-74, 1997, copy enclosed). The application discloses that the Tabby and EDA1-II protein sequences are 94% identical (see FIG. 1).

In any event, the USPTO does not require human data to demonstrate that a method will work as described. MPEP § 2107.03 specifically states that "[o]ffice personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials." Furthermore, MPEP § 2107.03 states that "data from in vitro or animal testing is generally sufficient to support therapeutic utility." Therefore, the USPTO must allow claims when data is presented showing a favorable result using the method in a laboratory animal.

It is incorrectly concluded in the Office action that Gaide and Schneider cannot be extrapolated back to the instant invention because the instant specification lacks support for the teachings of the references. The teachings of the present application provide support for the EDA1-II protein and methods used in Gaide and Schneider. Specifically, Gaide and Schneider provide an EDA1-II fragment (the C-terminal 147 amino acids of SEQ ID NO: 2), fused to another peptide. Although the present disclosure does not disclose this specific sequence, based on the teachings in the application, one could generate this sequence. For example, the present application discloses that the C-terminal 240 amino acids, and fragments thereof, are the biologically active domains of EDA1-II (see page 21, lines 21-25). In addition, the present disclosure teaches that EDA1-II proteins can be fused to other proteins (see page 15, line 29-page 16, line 16). Methods for generating such proteins using standard molecular biology methods are known in the art, and are disclosed in the application (for example see Example 22

starting on page 58). In addition, the present application provides support for the methods of treating the Tabby mice used in Gaide and Schneider. Gaide and Schneider injected pregnant mice intravenously with 2 mg/ml of protein, or newborn mice intraperitoneally with 40-100 μg protein. The present disclosure teaches administration of EDA1-II proteins intravenously or intraperitoneally (see page 72, line 22), at a dose of 0.01 mg/kg body weight to about 1 g/kg body weight (see page 15, lines 10-12) or at 1 ng/ml or 1g/ml (see page 51, lines 19 and 30). The average weight of a mouse is about 25 g (0.025 kg), so administration of 40-100 μg (0.04 – 0.1 mg) of protein is about 1.6 – 4 mg/ kg body weight. Therefore, the doses used by Gaide and Schneider were disclosed in the application. In summary, the teachings in the specification do not differ from that of Gaide and Schneider, and the results shown in Gaide and Schneider provide evidence that the claimed method works as described in the specification.

It is also incorrectly stated on page 7 of the Office action that neither the specification nor prior art teach one how to treat ectodermal dysplasia by introduction of EDA1-II as neither the specification nor the prior art provide dosages of EDA1-II to administer to patient, schedule of treatments, specific modes of administration of EDA1-II to humans suffering from ectodermal disease. As discussed at length above, this information is provided in the present application. For example, exemplary dosages of EDA1-II to administer to patient are provided on page 15, lines 10-12 (0.01 mg/kg body weight to about 1 g/kg body weight), an exemplary schedule of treatments is provided in Example 19 at page 50, lines 27-34; page 51, lines 6-7, line 20 and line 31 (daily, over a period of 6 weeks, 1-7 days, 1-3 months), specific (and exemplary) modes of administration of EDA1-II to humans suffering from ectodermal disease are provided in Example 29 at page 72, lines 21-37 (intradermal, intramuscular, intraperitoneal, intravenous, topical, and so forth). Therefore, the present application provides sufficient teaching to enable those skilled in the art to practice the claimed method.

In summary, Applicants have provided a detailed correlation between the structure of EDA1-II and the ability to induce development of ectodermal structures as well as information on how to identify EDA1-II fragments and variants that have the desired biological activity. In addition, the present application provides detailed teachings which enable those skilled in the art to practice the claimed method of increasing development of an ectodermal structure. In view of

the arguments presented herein, Applicants request that the 35 U.S.C. § 112, first paragraph rejections be withdrawn.

If any issues remain before a Notice of Allowance is issued, the examiner is invited to telephone the undersigned.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

By _____
Sheree Lynn Rybak, Ph.D.
Registration No. 47,913

One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, Oregon 97204
Telephone: (503) 226-7391
Facsimile: (503) 228-9446